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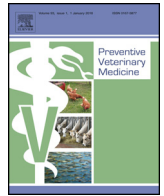
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A cross-sectional field study on potential associations between feed quality measures and usage of antimicrobials in commercial mink (*Neovison vison*)

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ABSTRACT

Feed quality is generally assumed to affect health status in animal production. In previous studies, the feed producer has been found to affect the occurrence of gastrointestinal disease and antimicrobial use in Mink (*Neovison vison*). Mink are fed with moist, freshly produced feed, based on perishable ingredients. The objective of this study was to investigate the potential effect of specific feed parameters on antimicrobial use on herd level. The study was cross-sectional, including 1472 mink herds, responsible for 97% of oral antimicrobials prescribed for Danish mink during the study period, 2012–2014. Data were obtained from the national veterinary prescription database (VetStat), Copenhagen Fur database, and the Voluntary Feed Control (Mink producers Organization). All feed batches subject to feed control were included. A multi-variable variance analysis was carried out analysing the effect of the feed parameters total volatile nitrogen, dry matter, crude protein and fat; total bacterial count (21 °C), and counts of sulphite producing bacteria (21 °C), *Clostridium* spp., faecal cocci (FC) (44 °C), yeast, and mould; presence of *Salmonella* spp. and *Clostridium perfringens* (dichotome). Three outcome variables were applied: prescription of oral antimicrobial on herd level within time slots of 3, 5 or 7 days after feeding of an included batch. Two binomial models were developed, adjusting for significant effects ($p < 0.0001$) of *Ps. aeruginosa* infection, herd size, month (season) and year. Antimicrobial prescription was significantly ($p < 0.0001$) associated with FC (all time slots, both models). A negative association ($p < 0.0001$) with crude protein on antimicrobial prescription within a 7 day slot suggested an association between low content of crude protein and antimicrobial use. The associations need to be confirmed in controlled studies, and ideally, potential causalities should be investigated. The perspective of such findings could be the development of tests for control of feed ingredients prior to use in the feed production.

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1. Introduction

Feed quality is considered an important factor for the animal health in many livestock species, but field studies in this area are rare, due to a decentralized feed production (often on-farm mixing) in many production types. The American mink (*Neovison vison*) is used for livestock production and the pelt is traded on a global market as dried skin. In Denmark, 1465 commercial mink farms were registered in 2014, housing 3.3 million breeding females (Clausen, 2014). Feed producers supply the mink farms with freshly produced, moist feed on a daily basis (from mid-April to the beginning

of December) or every second day. The farms are continuously supplied from the same feed producer, resulting in a hierarchical structure with feed of same composition and quality within groups of farms supplied from a specific producer. The feed is mainly composed of products of animal origin (e.g. offal from the fish and slaughter industries), which are highly perishable products. This is a problem particularly in the summer month, coinciding with the susceptible post-weaning and growth period of the mink. In the northern hemisphere, the minks are mated in March; mink kits are born around the 1st of May, weaned at 8 weeks of age, and pelted in November. In the winter period, only the breeding stock will be housed on the farm and in Denmark breeding males will normally be pelted after mating.

Antimicrobials used for animals in Denmark must be prescribed by a veterinarian and prophylactic use is prohibited. However, the

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use of antimicrobials per animal produced gradually increased by 102% during 2007–2011 (Jensen et al., 2016) and since remained at a high level, for no obvious reason: There has been no documented increase in the number of outbreaks with specific pathogens, and no liberalization of the use. Antimicrobials are often used for treating unspecific diarrhoea or pneumonia, and only to a lesser extent for outbreaks of specific pathogens. A previous field study supported that laboratory confirmed outbreaks of *Pseudomonas aeruginosa*, astrovirus (diarrhoea), influenza virus and *Salmonella* spp. were significantly associated with antimicrobial use (Jensen et al., 2016). However, Jensen et al. (2016) also found a highly significant effect of feed producer on the antimicrobial prescription pattern on mink farm level, associating some feed producers with a higher antimicrobial use on the associated farms. An earlier study of gastrointestinal disorders in mink identified feed producers as a risk factor, accounting for an important part of the between farm variation of gastrointestinal disorders (Rattenborg et al., 1999). These studies supported that low quality or contaminated feed may cause disease outbreaks in the recipient mink farms, but the importance of specific factors remained unknown.

The aim of this study was to investigate the potential association between the various available feed quality measures and the prescription of antimicrobials in the Danish mink production.

2. Material and methods

2.1. Population under study and data inclusion

In Denmark, a farm is defined by an identity code (CHR-ID) within the Central Husbandry Registers (CHR). The CHR-ID was used to merge data from different sources. The data on the relation between the feed producer, CHR-ID, and the herd size was obtained from the registers at Copenhagen Fur, based on yearly reporting from members of the Danish Fur Association. The study period was 2012–2014 and the study included all mink farms complying with the inclusion criteria:

- 1) Only farms that were members of the Danish Fur Breeders Association and complied with the annual reporting were included, resulting in 1482 mink farms. The feed producer was given in these records, and each farm was associated with one of thirteen feed producers which were active throughout the study period. Based on these criteria, 118 herds receiving 2.9% of the antimicrobials prescribed for oral treatment of mink during the study period were omitted.
- 2) One of the feed producers was excluded because it was very small (supplying 10 farms) and voluntary feed control was performed on only 44 batches as compared to around 100 (84–109) batches from the other feed producers. The 10 omitted farms were responsible for 0.02% of all antimicrobials prescribed for oral use in mink.
- 3) One farm using home mixed feed was omitted. This farm was responsible for 0.5% of antimicrobial prescribed for oral use in mink.
- 4) Only prescriptions of antimicrobial within a 7 day period after a feed batch had been subject to feed control were included. Accordingly, 53% of antimicrobials prescribed for oral use in the study herds during 2012–2014 was excluded.

The resulting dataset comprised 1472 study herds, 12 feed producers, and 47.4% of all antimicrobials for oral use prescribed for mink in the study herds, and 45.8% of all antimicrobial for oral use in mink during the study period.

2.2. Data sources

2.2.1. Animal population and estimation of farm animal biomass

The data on the herd size for active herds in a given year was obtained from the registers at Copenhagen Fur. Some farms had associated summer farms, which must be presumed to be closed during December–April; this was accounted for during the data validation process and preparation of data.

As the animal biomass fluctuates significantly over the year, the biomass per farm on a given day was estimated for the descriptive analysis: The average weight of a dam and the progeny for a given day was estimated from growth curves for the mink kids (Anonymous, 2013), time of birth and pelting, and actual weight data from a sample of farms (Anonymous, 2015). The weight of the average female or male varies seasonally from a minimum weight in March, prior to breeding, to a maximum in November. From 2012 to 2014, the weight of the average male in November increased from 3.6 kg to 3.8 kg. Correspondingly, the maximum (November) weight of the average female increased from 1.95 kg in 2012 to 2.05 kg in 2014. The estimated monthly biomass was corrected for this variation. The average biomass on a farm related to each dam on a given day was estimated as

$$w_{jk} = d_{jk} + n_k * p_{jk} + a * m_{jk}$$

where d is the average weight of a dam on day j and year k ; n is the size of an average litter for a given year, and p is the average weight of a kit on day j and year k ; m is the average weight of adult males on day j , and year k ; a is the proportion of breeding males per dam.

The live biomass estimated on farm level on a given day was calculated as the number of registered breeding females multiplied by the average biomass, w_{jk} .

2.2.2. Disease diagnosis

The National Veterinary Institute (NVI), Technical University of Denmark is the national reference laboratory for fur animal diseases in Denmark. The carcasses and/or other diagnostic material submitted from veterinary practice are subjected to a standard necropsy protocol with subsequent relevant routine diagnostic tests. The data are considered to have a high coverage for *P. aeruginosa* outbreaks, because the farmers are compensated by Copenhagen Fur for losses due to this infection, only if the diagnosis is confirmed at the NVI. No compensation is available for other diseases and few veterinarians submit samples systematically to the NVI. Consequently, very few positive laboratory results were available on these pathogens. Furthermore, influenza virus and *Salmonella* spp. are often feed borne and outbreaks might be confounded with feed batches. Hence, it was decided to include only *P. aeruginosa* as a risk factor in this study. The full dataset contained 74 instances of a positive *P. aeruginosa* diagnosis within the study period, affecting 68 herds with five farms affected more than once. The dataset for the 7-day periods contained 32 positive diagnoses of *P. aeruginosa*, affecting 31 farms.

2.2.3. Feed quality

For each of the feed producers, a voluntary feed control is currently carried out through test of the ready-to-eat feed batches (between 23 to 29 samples from each feed producer in 2012) on a regular basis – in most instances on a monthly basis (Christensen et al., 2013). Data were obtained from the annual report of these data (Anonymous, 2015). For each sample, at least four analyses of nutrients and ten different analyses reflecting the microbiological quality were carried out according to standard procedures (www.danskpelsdyrfoder.dk/). The nutritional feed parameters comprised total volatile nitrogen (TVN), dry matter, crude protein (CP), and crude fat (CF); the microbiological parameters comprised

the total bacterial cell count (21 °C), and counts of sulphite producing bacteria (21 °C), *Clostridium* spp., faecal cocci (44 °C), yeast, and mould; finally, the presence (+/–) of *Salmonella* spp. and *Clostridium perfringens* was determined.

2.2.4. Antimicrobial prescription

Data on prescriptions of antibacterial medicines for mink were extracted from the national veterinary prescription database, VetStat (Stege et al., 2003). Each prescription is represented by a record, including information on date of purchase, product identity and quantity, farm CHR-ID, target animal species, target age group, target disease category, and the identity of the prescribing veterinarian. VetStat data are considered to cover more than 99% of the total prescribed amounts of antimicrobials for veterinary use (Anonymous, 2002). In the first step, all records on sales of antimicrobials for local gastrointestinal (GI) or systemic treatment prescribed to mink farms (based on the CHR-ID) were extracted from VetStat. In 1% of the records prescribed to mink farms, a valid animal species was not given; these records were excluded unless the medicinal product was known to be used in mink and no other relevant species was recorded on farm. The amounts of antimicrobial were converted into number of defined animal daily doses (DADD) for treatment of one kg mink, as previously described (Jensen et al., 2016). Disease caused by low feed quality is most likely treated by orally administered drugs. Therefore, only oral medication was included, accounting for 97.5% of all antimicrobial (in DADD) prescribed for mink. Of the antimicrobial for oral use, 47% was used in the study herds within the 7-day periods relating to the included feed batches (see Section 2.4.1); this final dataset comprised 8379 pharmacy records and 820 records from the veterinarians. The records from the veterinarian were validated for errors in the reported prescribed amounts; these may be created by the invoice systems of the veterinarian practices, as multiplication or division of the prescribed amounts by package size; no such obvious errors were found in data on oral use in the study period.

2.3. Statistical methods

Data were organized, validated, and analysed using the software SAS®, version EG 6.1 and version 9.4.

2.3.1. Descriptive analysis

For the descriptive analysis, the monthly treatment incidence defined as number of DADD/biomass-days (Jensen et al., 2016) was estimated at the national level: The number of monthly biomass-days was estimated for each farm by summing up the estimated live biomass (Section 2.2.1) for each day. The temporal trend in oral treatment proportion on the national level was calculated for all mink herds in the registers of Copenhagen Fur (Section 2.1) on the national level. For the descriptive analysis, the average treatment proportion in the 7-day periods (relating to feed batches) across all study herds was calculated as

$$\text{Treatment proportion, } TP_{jk} = \sum ND_{abjk} / \left(\sum (\text{live biomass} * \text{days}_{abjk}) \right)$$

where ND_{abjk} is the number of DADD, prescribed for each farm, a , in any given 7-day period (see Section 2.3.2), b , month, $j = [1–12]$, and year, $k = [2012–2014]$. Here the denominator was the live-biomass-days within each 7-day period summarized on month and year across farms.

2.3.2. Study design and description of variables

The study sample was cross sectional, including all feed batches with related feed control analyses, and all the recipient farms (according to the hierarchical structure between farms and feed producers registered at Copenhagen Fur); thus, farms were

included as a random nested factor. Two different models were used in analysing the data; model A which was a logistic regression based on binary response data on antimicrobial use on farm level; and model B, also referred to as the batch model, which was based on aggregated data for each batch of feed. More details on the models are given in Section 2.3.3.

Three outcome variables were defined and modelled separately: antimicrobial prescription within a 3-day period, a 5-day period and a 7-day period in relation to a feed batch. The feed sampling day (day 0) was the day the feed was produced and delivered on the farm. Feed borne disease may occur as early as day 1 but more likely on day 2, whereas antimicrobial prescription would normally occur on day 2. Consequently, the observational time slots began on day 2. The initiation and duration of treatment may vary depending on whether disease is caused by an infectious outbreak, dietetic diarrhoea or simply poor food quality (such as high level of TVN, as an indicator of decomposed feed). Potential disease symptoms caused by the low feed quality in respect to one or more measures were expected to occur within a period of 1–3 days. However, the treatment may be further delayed due to the decision process of both farmer and veterinarian, which may depend on extent and severity of symptoms, which may in turn be related to the specific feed parameters related to disease. As treatment may be variably delayed, it was decided to model an array of outcome variables, i.e., recorded antimicrobial prescription within each time slot (J): 3 day period (2–4); 5 day period (2–6); and 7 day period (2–8) in relation to each feed sampling (day 0).

In some cases, analyses are performed on a new feed batch within a few days after the previous batch was analysed. This often occurs when feed quality breaches have been found in the previous batch. Consequently, time slots sometimes overlapped. In these cases, only the first feed batch related to the overlapping time slots was included in the final data set.

A descriptive analysis indicated that when prescription took place an average of 5.3 DADD per kg animal biomass on the farm were prescribed, corresponding to treatment of all animals on the farm for 5.3 day; 50% (25–75 percentile) of the prescriptions were for treatment of all animals (total biomass) for an estimated 3.5–9.2 days, or part of the herd for a longer period (Fig. 1). Consequently, the effect of feed quality in batches that was tested within the 3, 5, or 7 day time slot of a prior batch, was likely to be influenced by the feed quality of the prior batch. This was an additional reason for omitting the latest of the two feed batches, when time slots were overlapping, i.e. to avoid the interaction. Consequently, the number of observations was reduced with increasing length of the time slots.

In the vast majority observations, no antimicrobial was described and the proportion declined the shorter the time slot applied. For the 7-day period, antimicrobials were prescribed in 3.8% of the observations in the final data set (in 3714 of 97,566 feed batch – farm combinations). Due to the large number of zeroes, the outcome could not be modelled as a continuous variable, and a binary outcome was chosen.

In regard to the treatment of *P. aeruginosa* outbreaks, it was assumed that the antimicrobial was prescribed in close relation to the submission to the NVI. Therefore all positive diagnoses of *P. aeruginosa* (PA-disease) with a sample submission date within the given time slot (J) were included.

The potential explanatory variables were defined as follows:

Class variables:

- *Salmonella* spp. (0/1); *Clostridium perfringens* (0/1) – Feed quality measures (see Table 1).
- Month – the month of day 2 after feed sampling as a proxy for seasonal effects.

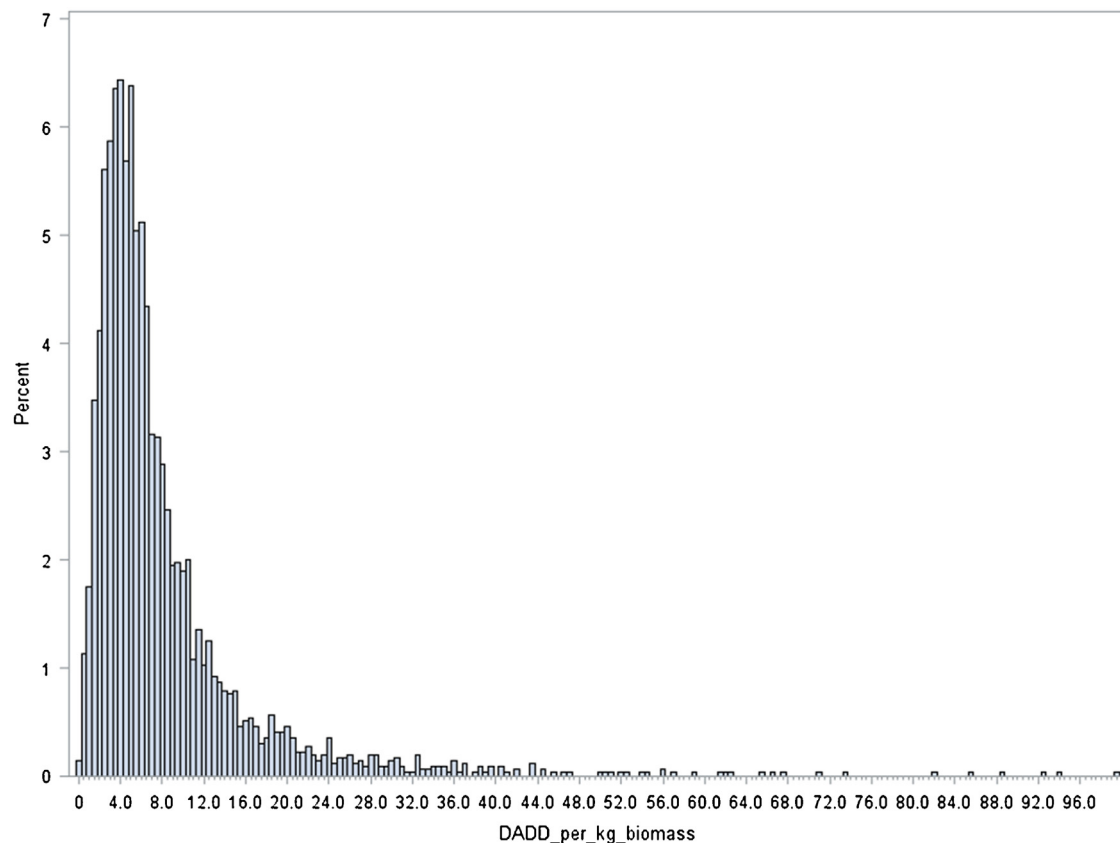


Fig. 1. Number of prescribed doses¹ per prescription relative to the number of animals.

1: Estimated as number of DADD (for one kg animal)/kg of live biomass.

Each observation includes prescriptions processed within a 7 day period after feeding the batches included in this study. A total of 3713 observations (number of farms*time slots, where prescription occurred) were included. One outlier observation (DADD/kg biomass = 147) was omitted from the figure.

Table 1

Descriptive statistics of feed quality parameters measured in 1176 batches of mink feed, 2012–2014.

Feed parameter (unit)	Abbreviation	Scale and model transformation	Mean	Median	Range ^a	SD
Total cell count, 21 ⁰ (g ⁻¹)	CC21	Continuous, log	1.6*10 ⁶	4.7*10 ⁵	5.0*10 ⁴ –6.1*10 ⁶	5.3*10 ⁶
Sulphite prod. bacteria, 21 ⁰ (g ⁻¹)	CC _{sulphite}	Continuous, log	8.1*10 ⁴	1.3*10 ⁴	8.0*10 ² –2.7*10 ⁵	4.6*10 ⁵
Fecal cocci, 44 ⁰ (g ⁻¹)	Fecal	Continuous, log	6.4*10 ⁴	4.2*10 ²	0–2.6*10 ⁵	3.0*10 ⁵
<i>Clostridium</i> spp. (g ⁻¹)	Clostridia	Continuous, log	1.3*10 ³	2.0*10 ²	0–4.2*10 ³	6.3*10 ³
<i>Clostridium perfringens</i>	CIP	Dichotomous	0.032 ^b	–	–	–
<i>Salmonella</i> spp.	Salm	Dichotomous	0.038 ^b	–	–	–
Yeast (g ⁻¹)	–	Continuous, log	1.9*10 ⁴	5.3*10 ²	1.2*10 ³ –8.9*10 ⁴	4.3*10 ⁴
Mould (g ⁻¹)	–	Continuous, log	1.6*10 ³	5.0*10 ²	0–5.0*10 ³	5.2*10 ³
Ash (%)	–	Continuous	3.3	3.2	2.3–4.5	0.7
Crude protein (%)	CP	Continuous	15.5	15.4	13.6–17.5	1.2
Crude fat (%)	CF	Continuous	8.3	7.7	5.1–12.2	2.5
Dry matter (%)	–	Continuous	37	37	31–43	4.1
Total volatile nitrogen (%)	TVN	Continuous	1.8	1.8	1.2–2.6	0.5
Acidity (pH)	–	Continuous	5.7	5.6	5.2–6.3	0.3

^a 5th–95th percentile.

^b The proportion positive feed batches.

- **Year** – the calendar year.
- **Herd size** – a categorical variable based on the number of dams (breeding stock) registered: small herds (<1500 dams), medium herds (1500–2499 dams), large herds (≥2500 dams).
- **PA-disease**; *P. aeruginosa* outbreak verified by laboratory analysis on samples submitted to NVI within the time slot related to the feed batch. In the batch model, the variable measured the number of farms with a positive *P. aeruginosa* diagnosis within the batch (*disease-count*). In the binary model, the diagnosis was related to the individual farm at a specific point in time.

Continuous variables:

Microbiological feed quality and nutritional feed quality parameters as listed in Table 1.

Random variables:

Farms (CHR-ID) were included as a random effect to adjust for between-farm variations (Model A).

2.3.3. Modelling procedures

Two generalized linear models were developed using the GENMOD procedure in SAS[®], with herd level antimicrobial prescription

as the response variable. In both models, the response variable was antimicrobial use on herd level within a defined time slot:

- Model A, the binary model: The dichotome (0/1) response variable, AM, represented antimicrobial use on herd level in relation to a given feed batch. The model was a logistic regression model, fitting the data to a binomial distribution.

$$AM = ash + Drymatter + TVN + CF + CP + \log(mould) \\ + \log(yeast) + \log(fecal) + \log(clostridium) + \log(CC) \\ + \log(CC_{sulphite}) + CIP + Salm + herdsize + disease,$$

with farm (CHR-ID) as a random effect. The abbreviations for the microbiological parameters are explained in Table 1.

- In model B, the batch model: The response variable was the proportion of farms with antimicrobial use (positive trials) out of the number of farms receiving the feed batch (trials). The data was fitted to a binomial distribution in a logistic regression model:

$$\frac{AM_{posfarms}}{no.offarms} = ash + Drymatter + TVN + CF + CP \\ + \log(mould) + \log(yeast) + \log(fecal) + \log(clostridium) \\ + \log(CC) + \log(CC_{sulphite}) + CIP + Salm + \overline{herdsize} + \overline{disease}$$

where $\overline{herdsize}$ and $\overline{disease}$ are mean values of the class variable for the farms receiving the same batch of feed. The abbreviations for the microbiological parameters are explained in Table 1. In the batch model, over-dispersion occurred and a scale parameter was set equal to the Pearson deviance.

In model A, the direct linking of the farms' AM value to the farm specific parameters *herd size* and *disease* was an advantage, whereas in model B the farm specific parameters were included in the model as mean values over all the farms receiving the same batch of feed. The advantage of model B, was that all farms given the same batch of feed were evaluated together. Analysis of the correlation structure of all feed quality variables was calculated using the pairwise Pearson correlation coefficients (PROC CORR in SAS). The results showed that *crude fat* and *dry matter* was highly correlated ($r=0.89$); consequently, it was decided to exclude *dry matter* from the analysis. All other feed parameters were included as potential explanatory variables, together with *herd size*, *month* and *year*.

To investigate the distribution of the continuous variables, each feed parameter was grouped into 25 percentiles and model A was run, including *herd size*, *year*, *month*, *PA-disease* and one feed parameter at a time; each feed parameter was plotted against the outcome variable adjusted for *herd size*, *year*, *month* and *PA-disease*. Based on these plots it was decided to include the nutritional parameters as continuous variables and to log-transform the microbiological parameters.

Antimicrobial use is generally very low in December and January, and the data for these two months (within the same year) were joined to achieve an adequate number of observations to enable modelling.

Every farm has a particular associated veterinarian according to current legislation, attending the farm with a minimum of 4 yearly mandatory visits, and a significant effect of the associated veterinarian on level of antimicrobial use has previously been demonstrated (Jensen et al., 2016). In the previous study, there was no interaction or confounding between feed producer and veterinarian. In the present study, the model could not run with both veterinarian and feed components in the model simultaneously; The veterinarian was therefore regarded as a characteristic of the individual farm (similar to management), as the objective was to investigate the effect of the feed. Thus, Model A adjusted for the effect of veterinarian by inclusion of *farm* as a random effect.

Significant effects of feed producer on gastrointestinal disease (Rattenborg et al., 1999) and antimicrobial use (Jensen et al., 2016) has been demonstrated previously. In this study, we assumed that the effect of feed producer was due to differences in feed quality, i.e. some feed producers have better feed quality than others. To avoid confounding, *feed producer* was not included in the model, because we wanted exclusively information about the feed parameters rather than the (indirect) predictive effect from feed producer. Due to the large number of farms the variable *farm* was included in the model as a random variable.

The models were fitted by stepwise backwards elimination procedure. A conservative significance level was set at 0.001 for both models due to the magnitude of the dataset.

3. Results

3.1. Descriptive analysis

On the national level, the treatment proportion decreased by 42% from 48 DADD/1000 kg biomass in 2012 over 36 DADD/1000 kg biomass in 2013, to 29 DADD/1000 kg biomass in 2014. In the 7-day period of the study herds, a similar seasonal and temporal trend over the years was observed (Fig. 2).

The annual number of feed batches analysed was almost unchanged during the study period, increasing by only 2.5% from 2012 to 2014. Fig. 2 shows the temporal trend in treatment proportion at the national level and for the included data for the 7-day period.

The time slots of 3, 5 and 7 days included 27%, 40%, and 47%, respectively, of the total amount of antimicrobial prescribed for oral use in the study farms.

The duration of treatment may vary depending on extent, severity and symptoms. For the 7 day study-period, the median was 5.6 DADD/kg biomass and the mean was 7.9 DADD/kg biomass (corresponding to 5.6 and 7.9 daily doses per animal) with a wide distribution (Fig. 1). The 25 and 75 percentile limits were 3.5 and 9.3 DADD/kg biomass.

3.2. Multi-variable variance analysis

Year, *month*, and *herd size* were significant for all three time slots in both models. All models showed a significantly lower use of antimicrobials in 2014 compared to 2012–2013 (parameter estimate, $\beta=0.3$ for 2012 compared to 2014). The prescription of antimicrobial was significantly more frequent in May–August and in October compared to November, whereas the antimicrobial prescription was significantly less frequent in the period from December to March.

Ps. aeruginosa outbreak (not feed borne) is known to be associated with antimicrobial use, and accordingly, a confirmed diagnosis was found to be significant in the binary models with the 5 day-period and the 7 day-period; however, in the batch model the proportion of infected farms was significant only for the 7 day period (Table 2).

The results for the significant feed parameters are shown in Table 2. *Faecal cocci* was significant in all models, with little variation in parameter estimates. Mould was significant for antimicrobial use in the 3-day period for both batch and binary models, with a negative estimate, suggesting a “protective” effect. In the batch models (model B), all other feed parameters were non-significant. In the binary model (model A), 7 day-period, a significant interaction between log (faecal cocci) and *month* was found, suggesting an increased effect of faecal cocci in March, October and November. A similar trend, although non-significant ($p=0.08$), was

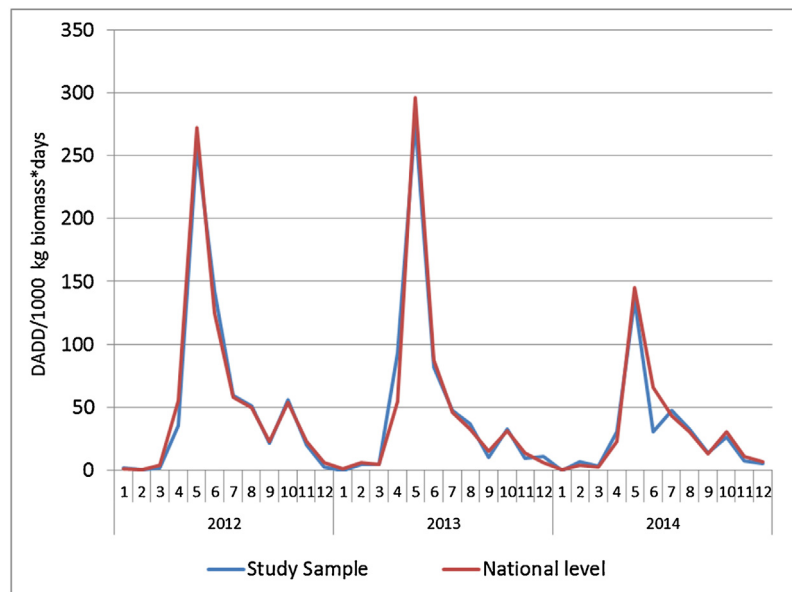


Fig. 2. Monthly treatment proportion for mink in the study sample and on the National level (DK) Treatment Proportion calculated as the number of daily doses (DADD) per kg-biomass*days.

Table 2

Parameter estimates for the effect of significant feed associated variables on antimicrobial use in 1472 mink herds.

Model type ^a , time slot	Log(faecal cocci/g) Parameter estimate [95% confidence interval]	Log(mould/g)	Percent	Log(faecal cocci/g)
A –Binary, 3 days	0.15** [0.09;0.21]	–0.15** [–0.20;–0.10]	ns	*
B –Batch, 3 days	0.14** [0.07;0.21]	–0.15** [–0.23;–0.08]	ns	ns
A –Binary, 5 days	0.14** [0.09;0.19]	*	*	*
B –Batch, 5 days	0.12** [0.06;0.17]	*	ns	ns
A –Binary, 7 days	0.13** [0.09;0.17]	ns	–0.08** [–0.12;–0.04]	**b
B –Batch, 7 days	0.11** [0.06;0.17]	*	ns	ns

Only the parameter estimates for the significant ($p \leq 0.0001$) feed variables are shown, because these were the only fixed effects included in the final models.

**Significant, $p \leq 0.0001$ *non-significant (borderline): $0.0001 < p < 0.02$; ns: non significant, $p > 0.02$.

^a Model A: outcome variable = antimicrobial use (0/1) on the individual herd. Model B: outcome variable = the proportion of herds with antimicrobial use among herds receiving the feed batch.

^b Interaction between *faecal cocci* and *month*, with significantly higher estimates for March, October and November.

observed in the batch model, 7 day-period, and in the binary models for 3 days-period and 5 day-period ($p = 0.01$).

4. Discussion

A very strong seasonal trend in antimicrobial use in mink has previously been observed, with significantly higher treatment proportions in relation to the whelping and weaning season in May–July and a minor increase in autumn, most likely related to respiratory infections (Jensen et al., 2016). In the present study, these seasonal trends in treatment proportion were also observed for the 2012–2014 period and found significant in the models. Furthermore, descriptive analysis showed that the treatment proportions in the subset of data included in modelling was almost identical to the trends observed in the full data on antimicrobial use. A significant decrease in frequency of antimicrobial prescription in the study periods was observed for 2014. Descriptive analysis showed a 42% decrease in oral antimicrobial use during the entire study period, reaching 29 DADD/1000 kg biomass in 2014.

However, the antimicrobial use remained at a much higher level than described for 2007–2008 in the previous study, with 21 DADD/1000 kg biomass*days (Jensen et al., 2016). Unpublished data for 2015 indicates that the decrease in 2014 was only temporary. The continued high level of antimicrobial use, which is comparable to the level in the pig production (Anonymous, 2016) stresses the need to identify causal factors.

Previous studies have shown that the feed producers were responsible for a large proportion of the variation in gastrointestinal disease in mink herds (Rattenborg et al., 1999) and the feed producer was a significant risk factor for the use of antimicrobial agents (Jensen et al., 2016). These findings strongly suggest an important role of feed quality on gastrointestinal disease and antimicrobial use in the mink production.

The present study demonstrated that increasing number of faecal cocci in the feed is associated with the risk for prescribing antimicrobial to the farms. This finding is particularly strong because count of faecal cocci was significant in all six fitted models. In general, the parameter estimates were slightly higher for

the binary model compared to the batch model; this suggests that herd related factors, affect the farm level response to inferior feed quality; herd related factors could be the associated veterinarian, farmers threshold for treatment, or management factors affecting the development of disease; the veterinarian has previously been found to have significant effect on antimicrobial use on farm level (Jensen et al., 2016).

In the binary model with an observational time slot of 7 days, a significant interaction between faecal cocci and month was found, suggesting an increased importance of faecal cocci in March and in October–November. In these months, feeding intensity is particularly high, due to either flushing of the dams (March) before mating, or an attempt to increase body mass before pelting (November). A similar trend was observed in the batch model (7-day period); again, this was not significant in the batch model, suggesting variation between farms in the effect (or response) to low feed quality.

Our previous study (Jensen et al., 2016) suggested that a microbiological feed score had an effect (borderline, $p=0.002$) on amounts of antimicrobial prescribed, independently from the effect of feed producer. The score was based on four measures, i.e. total bacterial cell count (21^0), sulphide producing bacteria (21^0), faecal cocci (44^0), and mould. The present study suggests that the effect of score was due to the underlying count of faecal cocci. With regard to mould, the present models show a negative parameter estimate for the 3-day period. This is likely an incidental finding; to our knowledge there is no evidence to suggest that mould could be beneficial for gastrointestinal health. Furthermore, mould was significant only for the 3-day period, supporting that it may be an incidental finding.

The binary model suggested an effect of crude protein on the antimicrobial use within the 7-day period. This may also be an incidental finding. However, if low content of crude protein is a true risk factor, the results suggest that the potential negative health effects would be protracted, because the significant increase in treatment appears to be delayed relative to feeding (significant in the 7-day model only). Further, the estimated effect of crude protein was of the same magnitude but not significant in the batch model, 7-day period ($p=0.07$; data not shown).

PA-disease was significant in the binary model, 5-day period and in both models for the 7-day period. A likely explanation is that the number of positive diagnoses was higher for the 7-day period; also, it may indicate that treatment of *P. aeruginosa* often occurs with a time delay relative to the sample submission. *P. aeruginosa* is not a feed borne infection, and therefore it is not likely that the effect on antimicrobial use has any relation with time after feeding a particular batch. *Salmonella* Dublin in the feed is known to cause abortion storms in mink (Dietz et al., 2006), whereas but infections with of *Salmonella* from the feed outside the gestation period may only be associated with diarrhoea. Previously, a positive diagnosis of *Salmonella* infection was found to be borderline significant for the use of antimicrobial on herd level (Jensen et al., 2016). No significant effect of in feed *Salmonella* was found in the present study. Possibly this was due to the low incidence of *Salmonella* spp. in the feed (3.8% of the batches), and part of these may be non-pathogenic strains. This suggests that spread of salmonella by feed is not a major problem in regard to antimicrobial use, possibly because antimicrobials are not used in relation to abortion storms. Also, clinical salmonellosis has been found to be uncommon in mink, even in a population where salmonella was common in mesenteric lymph nodes (Williams and Bellhouse, 1974).

The over-dispersion in the batch model indicated the presence of explanatory factors associated with the batch that were not

explained by the model; this could be other feed parameters that are not included in the analyses.

In conclusion, the present study supports that feed quality is important for maintaining health in the mink production. The results suggest that analysis of the content of faecal cocci in the feed could potentially be used for quality control of feed ingredients, ensuring either condemnation or sufficient heat treatment of ingredients before including it in the feed for mink. However, the results need to be confirmed in controlled studies, and ideally, potential causalities should be investigated. The present study suggests that other feed parameters, not routinely analysed for in the current system should be investigated for possible influence on the use of antimicrobial. The potential effects of mould and low content of crude protein, as suggested by the present study need further studies. The development of rapid methods for detection of faecal cocci or more specific pathogens like influenza and *Salmonella* Dublin could be relevant when raw products without heat treatment are used in the feed production.

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